SUPPORT FOR THE AMENDMENTS

Claims 2, 3, 8, 10, 15, and 21-26 are canceled.

Claims 1, 5-7, 11, and 16 are amended.

The amendments to Claims 1, 5-7, 11, and 16 serve to improve clarity and to improve readability. The amendment to Claim 1 finds support in original Claims 1-3. All of the fluorescent derivation reagents disclosed in the present application are fluorescent reagents which are non-fluorescent themselves that are "fluorogenic". The amendment to Claim 16 is supported by Claim 21. The amendments to Claim 5 are found in original Claim 5 and throughout the specification.

No new matter is believed to have been entered.

REMARKS

Claims 1, 4-7, 9, 11-14, and 16-20 are pending in the present application.

Applicants thank the Examiner for the indication that Claim 21 is allowable (see page 11 of the Office Action). Consistent with this recognition, Applicants have amended Claim 16 to include the limitations of allowable Claim 21. Accordingly, the rejections of Claims 16-20 under 35 U.S.C. §103(a) over Patricelli (US 7,179,655), with or without Srinivasan (US 2007/0065343). With respect to these rejections as they pertain to the previously presented claims, Applicants make no statement as to the propriety of the same. The amendment herein to Claim 16 is solely to expedite allowance of the subject matter recognized by the Examiner to be allowable. Withdrawal of the rejections of Claims 16-20 under 35 U.S.C. §103(a) over Patricelli (US 7,179,655), with or without Srinivasan (US 2007/0065343), is requested.

The rejections of:

(a) Claims 1-7, 9, 15, and 26 under 35 U.S.C. §102(e) over Patricelli;

(b) Claim 10 under 35 U.S.C. §103(a) over Patricelli in view of Goodlett (US 6,629,040);

(c) Claims 11 and 12 under 35 U.S.C. §103(a) over Patricelli in view of Andersson (US 6,653,625);

(d) Claims 13 and 14 under 35 U.S.C. §103(a) over Patricelli in view of Toyo'oka (Anal. Chem. 1984, 56, 2461-2464),

are respectfully traversed.

As previously stated, an important objective in the post-genome area is the detection of trace amounts of expressed protein/peptide expressed through genes, and the separation and identification thereof.

In the past, peptide fingerprinting following two-dimensional electrophoresis was commonly used to achieve this objective. However, this method had problems with reproducibility of the method due to the complex procedure. Separation and identification methods using multi-dimensional high-performance liquid chromatography (multi-dimensional HPLC), and techniques using ICAT have recently been proposed to overcome this problem.

Among these methods, methods for separating and identifying protein/peptide directly by multi-dimensional HPLC have the shortcoming of requiring considerable labor and time since all proteins/peptides are processed simultaneously.

In addition, methods using ICAT attempt to comprehensively analyze protein/peptide by labeling the thiol groups of thiol-containing protein/peptide with an isotope-coded affinity tag (ICAT) reagent, capturing the protein/peptide with a biotin-coupled column, subjecting all of the proteins/peptides to enzymatic hydrolysis, separating the resulting mixture of peptide fragments by HPLC, and carrying out mass spectrometry on the peptide fragments with a mass spectrometer (MS).

However, since this method involves subjecting all thiol-containing protein/peptide to enzymatic hydrolysis, it has the shortcoming of fragments of non-target protein/peptide present in large amounts impairing detection and identification of target trace protein/peptide, thereby creating the need to achieve further improvement in this technical field.

With the foregoing in view, as a result of conducting extensive research for the purpose of radically solving the above-mentioned problems of the prior art, the inventors of

the present invention found that, differing from methods of the prior art, trace expressed protein and/or peptide, unable to be detected with methods of the prior art, can be detected and identified with high sensitivity by performing the claimed method.

The main feature of the present invention is "the detection of intact protein itself" and not "the detection of proteolytically digested protein" as described in Patricelli's invention (column 2, line 47 – 49, column 2, line 59 – 62, column 3, line 6 – 11, column 3, line 40 – 45, column 4, line 10 – 12, column 4, line 16 -18, column 4, line 52 – 54, column 4, line 64 – 67 and column 5, line 23 – 25). The term of "detection" in the present invention means "detection of existence and/or detection of the amounts of protein".

In the present invention, without any prior proteolytic digestion, the fluorescently labeled intact protein should be directly subjected to HPLC for separation to each labeled protein, the existence and/or the amount of which are/is fluorometrically detected (HPLC/fluorescence detection).

This concept is entirely different from the Patricelli's invention, in which "the detection of the proteolyzed peptides is greatly concerned" (column 2, line 54 – 56, column 2, line 62 – 65, column 3, line 6 – 14, column 3, line 40 – 47, column 4, line 31 – 33, column 4, line 52 -54, column 4, line 64 – 67, column 5, line 23 – 30, column 20, line 35 -37, column 20, line 41 – 44 and column 26, line 42 – 46), and "to achieve the performance of the separation, the steps of the concentration and fractionation are required before separation".

The amount of each protein in a sample thus detected in the present invention can be compared with that of the corresponding each protein in another sample to find out differences of the expressed proteins in the two samples.

Accordingly, several our published papers, utilizing the present invention, have demonstrated for the first time many differently expressed proteins related to early

Parkinson's disease from normal (Ichibangase T. et al. *Biomed. Chromatogr.* 2008; 22: 232-234), related to liver disease in hepatocarcinogenesis mice from normal (Ichibangase T. et al. *J. Proteome Res.* 2007; 6: 2841-2849), related to aging in rat hippocampus (Asamoto H. et al. *J. Chromatogr. A*, 2008; 1208, 147-155) and related to running speed of horses (Ichibangase, T and Imai, K. *J. Proteome Res.* 2009; 8, 2129-2134).

Furthermore, it was for the first time demonstrated that the exclusive expression of Ran-specific GTPase-activating protein (RanGAP) and peroxiredoxin-1 in the breast cancer cells, and tropomyosin-1 as solely expressed protein in the normal epithelial cell (Imai K. et al. *Biomed. Chromatogr.* 2008; 22, 1304-1314).

These findings could not be accomplished by the invention disclosed in Patricelli.

The second feature of the claimed invention is that the labeling reagent such as 7-chloro-*N*-[2-(dimethylamino) ethyl]-2,1,3-benzoxadiazole-4-sulfonamide (DAABD-Cl) should be "fluorogenic" that produces a fluorescent conjugate after reaction with protein and is non-fluorescent itself. Otherwise, the excess reagent in the labeling reaction interferes the HPLC-fluorescence detection of the amounts of existed labeled proteins in a sample.

This requirement is extremely different from Patricelli (see column 11, line 62 – 67, column 12, line 1 – 67, column 13, line 1 – 67, column 14, line 1 – 30 and column 23, line 51 – 52), wherein all the reagents (ABP) are not fluorogeic reagents but fluorescent reagents that produce fluorescent conjugates after reaction of proteins, and at the same time the reagents themselves are fluorescent.

In the present invention, the reagents like those in Patricelli are not usable because of the great interference of the excess reagents in HPLC/fluorescence detection. If those reagents are used in the present invention, the removal step of the excess reagents should be required as described in Patricelli (column 25, line 59 – 60 and column 26, line 24 – 29).

Thus, the reagents used in Patricelli are useless in the present invention. Moreover, the reagent in the present invention does not require any enrichment of the labeled protein such as described in Patricelli (column 2, line 45 -46).

The third feature of the present invention is that any proteins in a sample should be targeted, that is, proteins in a sample should be indiscriminately labeled with our fluorogenic reagent for HPLC/fluorescence detection.

In contrast, in Patricelli active proteins are exclusively the target proteins (column 2, line 35 – 41, column 2, line 50 – 53, column 3, line 4 – 6, column 3, line 16 – 25, column 3, line 27 – 29, column 3, line 30 – 33, column 3, line 36 – 40, column 5, line 4 – 13, column 5, line 23 – 25, column 6, line 44 – 46, column 7, line 4 – 27, column 7, line 30 – 35, column 15, line 53 – 57, column 15, line 59 – 67, column 17, line 40 – 44 and column 17, line 65 – 67).

In view of the foregoing, Applicants submit that the claimed invention is not anticipated by and/or obvious in view of Patricelli. Moreover, none of Goodett, Andersson, Toyo'oka, and Srinivasan compensate for the aforementioned deficiencies in Patricelli. Accordingly, the claimed invention would not be obvious even if Patricelli were combined with these secondary references.

Thus, Applicants request withdrawal of these grounds of rejection.

Applicants submit that the present application is now in condition for examination on the merits. Early notification of such action is earnestly solicited.

Respectfully submitted,

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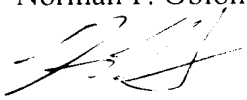

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